

# Comparative physical and immunological aspects of the chimpanzee and guinea-pig subcutaneous chamber models of *Neisseria gonorrhoeae* infection

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**SUMMARY** Physical and immunological characteristics of the chimpanzee and guinea-pig subcutaneous chamber models for *Neisseria gonorrhoeae* infection were compared to evaluate their usefulness for gonococcal research. Urethral infection in chimpanzees anatomically resembled the human infection; however, individual variation in response, limited availability, and the presence of interfering micro-organisms in the urethra were found to limit the usefulness of the chimpanzee in immunological research. Although the guinea-pig subcutaneous chamber model may not be suitable for studying the attachment of gonococci to host cells or for the local production of IgA, it does have the immunological advantages of being more sensitive to infection, less variable in response, free of interfering micro-organisms, and is readily available to investigators. Except for differences in sensitivity and variability, results with the guinea-pig model paralleled results obtained in experiments with chimpanzees. Unlike chimpanzees, guinea-pigs are a comparatively inexpensive, rapidly replenishable animal, which after subcutaneous implantation with small porous chambers provide a convenient model for studying most immunological aspects of gonococcal infections.

## Introduction

Limitations on the use of humans in medical research have stimulated interest in finding animal models in which human diseases can be studied. To be an effective immunological tool, an animal model must be readily available in genetically uniform groups and must be immunologically as similar to humans as possible. Additional factors of cost, accessibility, and humanitarian concerns must also be considered.

Throughout the past century, many attempts to transfer gonorrhoea from infected humans to animals have been recorded. A variety of inoculation techniques and test animals were used, but no reproducible animal model was developed (Hill, 1944). A procedure has been reported for infecting the eye of rabbits by inoculating gonococci into the anterior chamber, but the ensuing discomfort to the

animal and difficulty in recovering the infecting organisms restricted the use of this technique (Miller, 1948). The infection of chicken embryos with gonococci has provided researchers with some immunological information (Buchanan and Gotschlich, 1973; Bumgarner and Finkelstein, 1973); however, the chicken embryo may not be an appropriate model for studying complement mediated or other immunological systems absent in embryos. In a number of immunological studies recently reported, either the chimpanzee (Lucas *et al.*, 1971) or the guinea-pig (Arko, 1972) model of gonococcal infection was used. Although urethral infection of male chimpanzees with gonococci is the animal model which most closely resembles the disease in humans, the use of chimpanzees in large research projects is restricted. Therefore, a continuing effort is being made to determine the advantages and limitations of using the guinea-pig subcutaneous chamber model of gonococcal infection in the immunological study of *Neisseria gonorrhoeae*. The purpose of this report is to analyse recent experimental data and to provide additional information

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regarding the immunological and physical advantages, disadvantages, and problems encountered in using the chimpanzee and guinea-pig subcutaneous chamber models of gonococcal infection.

### Materials and methods

Intense interest in the immunology of *N. gonorrhoeae* combined with the development of practical infection models in animals has resulted in a recent flurry of experiments designed to study the serological, immunological, and pathological characteristics of this disease. The laboratory methods used for growing, identifying, and quantitating gonococci as well as the procedures used in preparing, infecting, and culturing experimental animals are described in the literature cited.

### Results and discussion

#### CHARACTERISTICS OF GONOCOCCAL INFECTIONS IN CHIMPANZEES

The report in 1971 of gonococcal infection in male chimpanzees after urethral inoculation with human exudate (Lucas *et al.*, 1971) stimulated interest in using this model for studying immune responses induced by experimental gonococcal infection. More recent studies involving chimpanzees have established the following main points: 1. Urethral infection can be induced in chimpanzees by inoculating them with cultures of gonococci grown on laboratory media, and male to female transmission can occur in sexually active cagemates (Brown *et al.*, 1972); 2. Urethral infections left untreated may persist for two months or longer, and serum antibodies to gonococci may be detected within three weeks after inoculation (Brown and Lucas, 1973); 3. A substantial increase in strain-related immunity to urethral infection may occur after parenteral immunisation with a formalin-killed whole cell vaccine (Arko, 1974); 4. A primary, untreated, urethral infection may, upon spontaneous remission, increase resistance to reinfection with the same gonococcal strain (Kraus *et al.*, 1975); 5. An enhanced resistance to homologous gonococcal infection may persist for two years or longer after systemic immunisation; serum antibody levels measured by the indirect fluorescent antibody and complement-dependent bactericidal tests correlate most closely with increased resistance to infection (Arko *et al.*, 1976b).

In the course of these immunological studies, other characteristics of the chimpanzee became apparent. A variety of different bacteria were found to colonise the urethra of male chimpanzees. *Proteus vulgaris* and other unidentified Gram-

negative micro-organisms were occasionally isolated from the urethra of chimpanzees being cultured for the first time. The incidence of these isolations generally increased with the frequency of urethral catheterisation used in the experimental challenge procedure. Although the influence of other micro-organisms on the subsequent susceptibility of the chimpanzee urethra to gonococcal infection was not conclusively determined, it is probable that the presence of these micro-organisms non-specifically increased resistance to urethral gonococcal infection.

Resistance to gonococcal infection, as observed with other diseases, is a multifaceted phenomenon which is primarily dependent upon the challenge dose and the immunity of the host. Other factors, however, such as the biological variation inherent in heterogeneous chimpanzees and the effects of other micro-organisms, can produce substantial variation in the resistance of normal animals to gonococcal infection. In three recent studies, the minimum infective dose (MID) of gonococci in 11 non-immunised male chimpanzees challenged in the urethra with graduated doses of M strain gonococci varied from  $1.8 \times 10^3$  to  $7.0 \times 10^5$  colony forming units (cfu) of virulent gonococci, with a geometrical mean MID of  $4.9 \times 10^4$  cfu. In these studies, gonococcal infections in male chimpanzees differed in some aspects from the disease observed in humans. Male chimpanzees inoculated with laboratory-grown gonococci did not develop a typical purulent urethritis as had been previously described for human volunteers inoculated with gonococci passed on laboratory media 700 times (Kellogg *et al.*, 1963, 1968). Also, the human virulent colony types (T) of two of four gonococcal strains were found non-virulent for the urethra of male chimpanzees who were subsequently infected with other gonococcal strains. These biological variations, as related to experimental results, have given rise to the question: Does gonococcal colonisation of the urethra or pathological infection occur in male chimpanzees? Until research can more fully elucidate gonococcal disease in chimpanzees, this will remain a concern.

In using chimpanzees for evaluating vaccine-induced immunity, the monitoring of three biological parameters was found to be important. Immunity in vaccinated animals appeared to be related to the challenge dose; therefore, to make a comparative evaluation, individual MID of gonococci must be determined for both the vaccinated animals and controls. Once the gonococcal infection has been initiated, it should be followed with serial cultures to its termination, since important differences in duration have been observed between infected vaccinated animals and control chimpanzees. In addition, the level of gonococcal colonisation in the

vaccinated and control animals should be culturally ascertained. The importance of these parameters is emphasised by the high degree of biological variation observed in the susceptibility of non-immunised chimpanzees to gonococcal infection. Although this variation is not unlike that which might be encountered in man, when the results of research studies are limited to a small number of subjects, as with chimpanzees, the degree of individual variation becomes critical.

The limited availability of the chimpanzee and the expense of conducting research with this animal have restricted the evaluation of its usefulness as an animal model in gonococcal research. Although female chimpanzees have been shown to be susceptible to cervical gonorrhoea by natural transmission, the experimental inoculation of the cervix and vagina has yielded highly variable results, with approximately half chimpanzees being resistant to infection. The mucous membranes of the oropharynx and ocular subconjunctiva are susceptible to gonococcal infection, but have been studied in relatively few animals (Lucas *et al.*, 1971). Rectal gonococcal infection has not been observed in male or female chimpanzees, even after experimental inoculation with  $10^8$  or more cfu of chimpanzee virulent gonococci. In addition, the qualitative or quantitative correlation of gonococcal colony types with virulence for chimpanzees has not been established. However, until a suitable alternative is developed, the chimpanzee remains the only animal model other than humans in which gonococcal infection of an epithelial surface can be studied.

#### CHARACTERISTICS OF GONOCOCCAL INFECTIONS IN SUBCUTANEOUS CHAMBERS IN GUINEA-PIGS

Small, porous, polyethylene or stainless steel chambers implanted subcutaneously in a variety of laboratory animals have been found susceptible to infection with *N. gonorrhoeae* (Arko, 1972, 1973). Although very different anatomically from the disease in man or the chimpanzee, the guinea-pig has been used to study the immunological aspects of different gonococcal strains and for testing the immunogenicity of potential gonococcal vaccines (Arko, 1974). However, whether this type of infection provides a valid model for use in studying human disease remains an important question.

Some aspects of this disease, such as the importance of gonococcal cell attachment to the host mucous membranes or the local effects of IgA, cannot be studied because of the absence of an epithelial surface in the subcutaneous chamber. There are, however, distinct advantages in using the guinea-pig subcutaneous infection model for studying certain

immunological phenomena of *N. gonorrhoeae*. Inbred lines of guinea-pigs are commercially available and relatively inexpensive; they alleviate the problems of biological variation and insufficient research subjects. The subcutaneous chamber is closed to the outside environment and therefore permits research to be conducted with controlled numbers and types of micro-organisms. Inocula can easily be injected or collected with a needle and syringe from these chambers with minimal discomfort or risk of contamination to the animal. In addition, this technique allows investigation of immunological responses and quantitative determinations of the bacteria and antibodies present at various intervals after inoculation.

Modification of the guinea-pig chamber implant and inoculation schedule, which produces variations in the degree of connective tissue encapsulation, results in changes in the chamber fluid complement concentration (Arko, Wong, and Logan, unpublished data). This, in turn, appears to affect the susceptibility and duration of gonococcal infections in subcutaneous chambers. The connective tissue capsule over the porous chamber becomes more dense with the length of time after chamber implantation. Chambers without the capsule, implanted for 10 days or less, generally contain a higher concentration of complement and are less susceptible to T3 gonococcal infection. In addition, infections in these chambers are of a shorter duration, lasting approximately two weeks with most strains of T1 gonococcal cells (Arko *et al.*, 1976c). On the other hand, chambers that have been implanted for 12 weeks or longer and have a dense outer capsule contain a lower concentration of complement and are more susceptible to the T3 gonococcal cells. Gonococcal infections in this type of chamber usually last much longer and vary more widely in their duration (Veal *et al.*, 1975). In both types of chambers, the development of resistance after infection or immunisation appears to correlate with the development of serum and chamber fluid bactericidal antibody to the homologous gonococcal strain (Scales and Kraus, 1974; Wong *et al.*, 1976).

A moderate degree of infectivity in subcutaneous chambers was found with fresh gonococcal isolates from clinical specimens. Approximately 80% (4/5) of the first inoculations produced infection when  $10^4$  or greater cfu of T1 cells were injected. The virulence of these isolates for guinea-pigs was greatly increased by animal passage. The median infecting dose for nine guinea-pig passaged gonococcal strains tested in 36 non-immunised guinea-pigs ranged from  $10^{1.3}$  to  $10^{2.7}$  cfu of T1 gonococci. In 12 similar guinea-pigs, a 100% infection rate was obtained after injection of  $10^{2.3}$  cfu of M strain gonococci.

This high degree of susceptibility to infection and uniformity of response makes the subcutaneous chamber a sensitive model for the testing of gonococcal strain relationships and for evaluating the effectiveness of immunisation with experimental vaccines.

The correlation of gonococcal colony types with virulence for guinea-pig subcutaneous chambers has been studied with five gonococcal strains. With three strains, cells from T1 colonies, but not T3 colonies, produced persistent guinea-pig infections. This correlates with the results in humans for a single strain (Kellogg *et al.*, 1963, 1968). Subcutaneous chamber infections with both T1 and T3 colony types have been observed with two other strains; however, a  $10^2$  to  $10^3$  times larger infecting dose was required with T3 cells than with T1 cells of the same strains. In addition, a gradual postinfection shift of predominance from T3 to T1 colonies occurred in serial cultures of T3 infected chambers, which suggests that a biological advantage for the survival of T1 gonococci exists in guinea-pigs. Differences in the susceptibility of the T1 and T3 cells to the bactericidal effects of 'normal' serum appear to provide a mechanism for the virulence variations observed in guinea-pig subcutaneous chambers.

Several studies have been conducted to determine the features of tissue disease and gonococcal cell wall structure that are characteristic of both human and subcutaneous chamber infections with gonococci. In one study, the tissue infections observed in subcutaneous chambers were similar to those of disseminated gonococcal infection in humans (Chandler *et al.*, 1976).

In electron microscopical studies of *in vivo* and *in vitro* cultured gonococci, the cell wall structure of gonococci obtained directly from the human urethra (Novotny *et al.*, 1975) was similar to that of gonococci obtained from guinea-pig and mouse subcutaneous chambers (Arko *et al.*, 1976a). There were, however, noticeable differences in the development of the peptidoglycan cell wall layer in both studies when *in vivo* and *in vitro* cultured cells were compared. Extensive *in vitro* cultivation of T1 cells seemed to produce changes in the peptidoglycan cell wall layer, and these cells became less resistant to the bactericidal effects of normal guinea-pig serum.

## Conclusions

In planning animal experiments, factors in addition to the technical aspects presented must be considered in determining the feasibility of using the chimpanzee or guinea-pig gonococcal models. Chim-

panzees, an endangered species, are heterogeneous, expensive, and slowly replenishable animals. Consequently, chimpanzees are frequently exchanged among different research centres. This requires that explicit identification and medical records be maintained for each animal, sometimes for 20 years or longer. For many animals, these records are incomplete or unavailable. Therefore, since more than 60 chimpanzees have been used in previous gonococcal studies, there is serious concern that the results of future work may be jeopardised by inadequate records of the previous immunological experience of research chimpanzees. There is also the increased risk of viral hepatitis and tuberculosis to investigators working with primate subjects. These problems with chimpanzee research are surmountable, however, provided that the resulting information warrants the effort and expense involved to obtain it.

The guinea-pig subcutaneous chamber technique is a relatively new approach for studying the infectious disease process; therefore, a complete evaluation of this model is not yet available. The available data indicate that this model is approximately 100 times more sensitive than chimpanzees to infection with the same strains of gonococci. Furthermore, with guinea-pigs fewer problems with individual variation are encountered and there is less likelihood that results will be influenced by previous gonococcal exposure or by contaminating micro-organisms. In addition, the guinea-pig is a comparatively inexpensive, quickly renewable animal that is universally available to investigators.

Improvement and standardisation of the chamber implant and the insertion technique should further increase the uniformity of immunological results with this model and provide a practical test animal for evaluating experimental gonococcal vaccine antigens. It must be stressed, however, that in the present form, this procedure should be used only by individuals trained in laboratory animal anaesthesia, surgical technique, and the humane treatment of research subjects. The subcutaneous chambers of biologically inert material should be prepared and designed to prevent the development of post-implantation pressure necrosis. The size and number of subcutaneous implants used in each animal may vary with the requirements of different experiments; however, they should not restrict the natural movements of the animal. The use of improper procedures can result in ulceration of the implanted chambers, followed by septic infection and generalised debilitation of the animal. Strict guidelines and close supervision are required to prevent the potential misuse and abuse of animals in this type of research.

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